Reference: *Biol. Bull.* **190:** 125–138. (February, 1996)

Efferent Mechanisms of Discharging Cnidae: I. Measurements of Intrinsic Adherence of Cnidae Discharged From Tentacles of the Sea Anemone, *Aiptasia pallida*

GLYNE U. THORINGTON AND DAVID A. HESSINGER

Department of Physiology and Pharmacology, School of Medicine, Loma Linda University, Loma Linda, California 92350

Abstract. Two kinds of cnida predominate in the tentacles of the acontiate sea anemones: spirocysts and microbasic mastigophore nematocysts. These cnidae discharge in response to appropriate mechanical and chemical stimulation. In this paper, we calculate the strengths of attachment between the tentacle and the capsules (= "tentacle adherence") of discharged spirocysts and mastigophores by measuring adhesive force and by determining the numbers of spirocysts and mastigophores discharged onto targets under conditions where the attachment of everted cnida tubules to the target (= "cnida adhesion") exceeds tentacle adherence. Under these conditions, the average contribution of individual cnidae to adhesive force is called the intrinsic adherence. The intrinsic adherence is a measure of the average frictional force required to dislodge the capsule of individual discharged cnidae from the tentacle. The intrinsic adherence of discharged mastigophores varies inversely, from 0.45 to 0.15 mgf (4.41 to 1.47 μN), with the number of discharged mastigophores. The larger values characterize mastigophores discharged by mechanically triggering nonchemosensitized tentacles, whereas the lower values characterize the intrinsic adherence of mastigophores discharged from chemosensitized tentacles. In contrast, the intrinsic adherence of discharged spirocysts is very low to insignificant. Thus, by comparison to mastigophores, spirocysts contribute little, if any, to adhesive force, and, by inference, do not directly secure captured prey to the tentacle. Our measurements indicate that penetrable prey are primarily secured to the tentacle by discharged mastigophores and by the inherent stickiness of the tentacle surface.

Received 17 January 1995; accepted 4 October 1995.

Abbreviations: CSCC, cnidocyte/supporting cell complex; S_t, tentacle stickiness; i_s, intrinsic adherence of spirocysts; i_m, intrinsic adherence of mastigophore nematocysts.

Introduction

Capture of prey

Active predators use remote sensing systems, such as vision or hearing, to detect and select prey over distance and to direct the neuromuscular movements needed to pursue and capture prey. Passive predators, on the other hand, do not usually select prey at a distance and do not pursue prey. They commonly rely upon local sensing systems, such as touch and chemical sensing, to detect and select prey in proximity and to elicit extremely rapid capture behaviors. Passive predation emphasizes rapid selection and responsiveness to the arrival of suitable prey. Cnidarians are obligate predators, and all, with the possible exception of the cubomedusae, are passive predators.

Feeding in sessile cnidarians, such as sea anemones, consists of sequentially linked prey capture, feeding response, and ingestion behaviors. In general, prey capture is initiated by appropriate stimulation of chemo- and mechano-receptors, and is culminated by cnida-mediated retention of prey to the tentacle (Thorington and Hessinger, 1988b). The subsequent feeding response, also activated by chemical and mechanical stimuli originating from the prey, is a coordinated series of neuromuscular movements that transfers captured prey to the oral disk and opens the mouth for ingestion to occur (Lenhoff and Heagy, 1977).

Afferent mechanisms regulate cnida discharge

While anemones and other cnidarians employ cnidae in various ways, cnidae are primarily used to capture prey (Ewer, 1947). We define mechanisms that regulate the initiation of cnida discharge (*i.e.,* receptor-effector coupling) as cnida afferent mechanisms. These mechanisms control the discharge of cnidae through the interplay of both local receptors associated with the cnidocytes that trigger discharge (Watson and Hessinger, 1988, 1991) and remote receptors that modulate cnidocyte responsiveness (as reviewed in Thorington and Hessinger, 1988b). In acontiate sea anemones, such as *Aiptasia pallida* and *Haliplanella luciae,* the functional and structural unit of cnida discharge is the cnidocyte/supporting cell complex (CSCC). In these anemones, two or more supporting cells surrounding each cnidocyte appear to bear at least one kind of chemoreceptor (Watson and Hessinger, 1988) and mechanoreceptor (Watson and Hessinger, 1991). When appropriately stimulated, two or more supporting cells surrounding each cnidocyte act upon the cnidocyte to initiate discharge.

We have identified three types of CSCC: Types C, B, and A (Thorington and Hessinger, 1990). Type C CSCCs are induced to discharge by sufficient physical contact. Type B CSCCs are induced to discharge by a combination of both contact and chemical stimulation. Chemoreceptors for free and conjugated N-acetylated sugars (*e.g.,* N-acetylneuraminic acid, NANA) and for certain amino compounds predispose contact-sensitive mechanoreceptors to trigger discharge (Thorington and Hessinger, 1988a, 1990). In addition, vibration-sensitive mechanoreceptors are associated with Type A CSCCs (Watson and Hessinger, 1989) and all three kinds of receptor are involved. Type A CSCCs discharge their cnidae in response to triggering contact stimuli following sensitization by vibrations at specific frequencies to which they can bidirectionally tune by stimulating antagonistic NANA (Watson and Hessinger, 1991) and proline chemoreceptors (Watson and Hessinger, 1994a).

Efferent mechanisms regulate cnida function

After the initiation of discharge, efferent mechanisms regulate the use of cnidae. As used in this report, the terms cnida adhesion, tentacle adherence, intrinsic adherence, and adhesive force have specific meanings associated with aspects of efferent mechanisms. Cnida adhesion refers to the interaction between everted cnida tubules and targets, and is measured as the force needed to separate the cnidae from the target. Tentacle adherence refers to the interaction between capsules of discharged cnidae and the surrounding tentacle to which they are anchored, and is measured as the force needed to remove discharged cnidae from the tentacle. The tentacle adherence of an individual cnida is called the intrinsic adherence. Intrinsic adherence is a measure of the frictional force required to dislodge the capsule of the discharged cnida from a tentacle. Adhesive force is a measure of the applied force needed to separate a target from the tentacle and is the sum of contributions arising from the discharged cnidae and the inherent stickiness of the tentacle mucus (Thorington and Hessinger, 1988a, 1990). When cnida adhesion exceeds tentacle adherence, contributions of discharged cnidae to adhesive force provide a measure of tentacle adherence. These relationships are shown diagramatically in Figure 1 and described later in Equation 1. Additional efferent mechanisms include the penetration of the target by everting nematocyst tubules and the delivery of nematocyst venom to the target. It is not yet known whether cnida efferent mechanisms are biologically regulated as are cnida afferent mechanisms. It is likely, however, that tentacle adherence is regulated, since prey attached to the tentacle by cnidae during capture are subsequently released from the tentacle during ingestion.

On the basis of the adhesive property of everted spirocyst tubules and the penetrating and venomous properties of mastigophores (Mariscal, 1974), it has been commonly inferred that penetrant nematocysts principally envenomate and immobilize prey, while spirocysts principally attach stung prey and inert substrates to the tentacles (McFarlane and Shelton, 1975; also, "Spirocysts are adhesive organelles which function both in prey capture and substrate attachment," Mariscal *et al.,* 1977). In other words, it has been commonly inferred that the intrinsic adherence of spirocysts exceeds that of penetrant nematocysts. On the other hand, we have recently presented qualitative evidence that mastigophore nematocysts predominate over spirocysts in securing penetrable targets to the tentacles (Thorington and Hessinger, 1990). However, until now, quantitative evidence for inferring the roles of mastigophores and spirocysts in the retention of prey has been limited to counting the numbers of discharged cnidae, but determination of their intrinsic adherence has been lacking.

As a part of our on-going investigation into the mechanisms that regulate cnida discharge, we have developed methods to quantitate discharging cnidae under conditions in which the eliciting stimuli and the target surfaces are defined. We now measure the tentacle adherence of cnidae under conditions in which cnida adhesion does not influence adhesive force (*i.e.,* when cnida adhesion exceeds tentacle adherence). From the measurements of tentacle adherence using monoclonal, laboratory-reared sea anemones (*Aiptasia pallida*), we infer the relative roles of mastigophores and spirocysts in securing captured prey. Our findings indicate that as long as mastigophores are

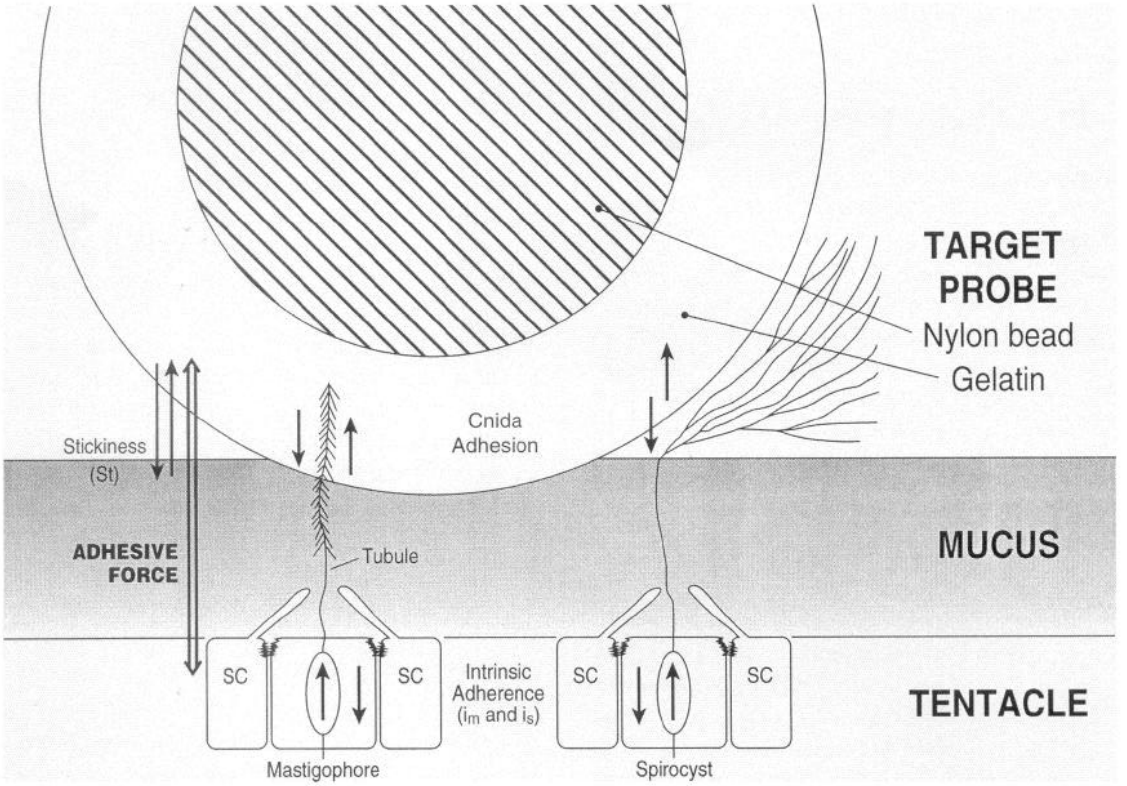

Figure 1. Relationship of adhesive force to cnida adhesion, intrinsic adherence, and tentacle stickiness. Double arrow represents the net opposing forces operating between the target probe and the tentacle, which is termed adhesive force. The individual opposing forces constituting adhesive force are: (i) cnida adhesion, operating between the target and the everted cnida tubule; (ii) intrinsic adherence (*i.e.*, i_m and i_s for mastigophore and spirocyst intrinsic adherence, respectively), operating between the tentacle and the cnida capsule; and (iii) stickiness (S_t), operating between the target and the surface mucus of the tentacle. When cnida adhesion exceeds tentacle adherence (the sum of all contributing intrinsic adherences), then the adhesive force is a function of the tentacle stickiness plus the sum of the intrinsic adherences of all the involved mastigophores and spirocysts. Diagram not drawn to scale. SC, supporting cell.

able to penetrate the surface of prey, cnida-mediated attachment of prey to tentacles is provided predominantly by mastigophore nematocysts, not by spirocysts. Thus, the function of spirocysts is in need of reevaluation.

MATERIALS AND METHODS

Maintenance of sea anemones

Monoclonal sea anemones (*A. pallida,* Carolina strain) were maintained individually in glass finger bowls containing natural seawater at 24 ± 1°C. Filtered seawater was obtained from the Kerckoff Marine Laboratory of the California Institute of Technology at Corona del Mar, California. Anemones were fed daily with freshly hatched brine shrimp nauplii (*Artemia salina*) and washed 4–6 h after feeding. Animals were kept on a 12/12 h photoperiod using white fluorescent lights at an intensity of 5.5 klux (66 μ Es^{-1} m^{-2}).

Experimental animals and test solutions

Animals of the same size were starved 72 h prior to experimentation and kept under continuous fluorescent light at 4.5 klux (54 μ Es^{-1} m^{-2}) during the last 48 h of starvation. Exposure to continuous light enhanced uniformity of anemone behavior and cnidocyte responsiveness. Just prior to use, the animals were gently rinsed with fresh seawater to remove soluble wastes and the medium was exchanged with the solution to be tested. The animals were permitted to adapt to the change of medium for 10 min before cnidocyte responsiveness was measured. Test solutions of various known chemosensitizers, N-acetylneuraminic acid (NANA), glycine, histamine, and proline (Sigma, St. Louis, MO) were prepared in natural, filtered (Type 1, Whatman) seawater adjusted to pH 7.62 with 1 *N* HCl or NaOH. Calcium-free artificial seawater (Ca-free ASW) consisted of 439 m*M* NaCl, 8.9 m*M* KCl, 23 m*M* $MgCl_2$, 22.5 m*M* $MgSO_4$, 4.6 m*M* $NaHCO_3$, and

1 m*M* EGTA (ethyleneglycoltetraacetic acid) at final pH of 7.62.

Measurements of cnidocyte responsiveness

Three measures of cnidocyte responsiveness were used: (i) measurement of adhesive force; (ii) enumeration of discharged mastigophores by microscopic examination; and (iii) enumeration of discharged spirocysts by enzyme-linked lectin sorbent assay (ELLSA).

Adhesive force. Adhesive force is a collective measure of both cnida-mediated and cnida-independent forces that act to retain a target surface to the tentacle. The measurement of adhesive force has been described in detail elsewhere (Thorington and Hessinger, 1988a). It employs a gravitometrically calibrated strain gauge to which is attached a gelatin-coated nylon bead of defined size (the test probe or target), which is touched to the distal third of a primary tentacle on an anemone submerged in seawater. The chemosensitizing agent may be presented to the anemone either in the seawater or in the gelatin-coating of the probe. Physically contacting the tentacle with the test probe mechanically triggers local discharge of cnidae. The everting tubules of the nematocysts and of the spirocysts, respectively, penetrate and stick to the gelatin coating. As the probe is pulled away, the discharged cnidae resist separation from the tentacle, causing an equal and opposite force to be exerted on the strain gauge. The measured adhesive force, which is recorded on a strip chart recorder, is that required to separate the probe from the tentacle. Adhesive force measurements are expressed in hybrid units of milligram-force (mgf), rather than newtons (or dynes), since there is negligible acceleration, thereby making contributions from Newton's Second Law insignificant (Miller, 1959).

Enumeration. After being used to measure adhesive force, the gelatin coating of the probe tips are enzymatically digested to release the discharged mastigophore nematocysts. The highly refractile mastigophores, which are resistant to proteolysis, are then counted with a microscope from the bottoms of flat-bottomed microtiter wells. The procedures used to remove and count the nematocysts have been published (Geibel *et al.*, 1988).

Enzyme-linked lectin sorbant assay (ELLSA). To determine the number of spirocysts discharged onto test probes, we used an enzyme-linked lectin sorbant assay, the details of which have been published by Thorington and Hessinger (1990). This assay is based upon the high-affinity binding of conjugated N-acetylated sugars to the everted tubules of discharged spirocysts on the surface of test probes. The assay involves colorimetric measurement of bound peroxidase activity using a microtiter plate spectrophotometer following the sequential treatment of test probes with solutions of asialomucin and *Vicia villosa* lectin/peroxidase conjugate.

Collection and analysis of cnidocyte response data

Separate animals were exposed to different concentrations of chemosensitizers in seawater. Six probes, one per tentacle, were used on each animal to measure adhesive force and to enumerate discharged mastigophores and spirocysts. Daily experimental means were calculated from these measurements and replicate experiments were performed on four days. Data points represent the mean of the daily experimental means ($n = 4$). In some cases, where indicated, previously published experimental values (Thorington and Hessinger, 1990) are used to calculate the contributions of individual cnidae to adhesive force measurements. Linear regression analysis and determination of extrapolated values for intrinsic adherence were performed with a computer-assisted graphics and data-formatting program (Dorgan and Hessinger, 1984).

Contributions to adhesive force

Adhesive force is a composite measure of contributions from discharged mastigophores, discharged spirocysts, and the stickiness of the tentacles due to its mucus layer. Under conditions where cnida adhesion exceeds tentacle adherence, the contribution of each kind of cnida to adhesive force is the product of the number of cnidae discharged and the force required to remove one such cnida from the tentacle (*i.e.*, intrinsic adherence). These relationships may be summarized by the following equation:

$$\text{Adhesive force} = S_t + (i_m)(n_m) + (i_s)(n_s) \quad \text{(Equation 1)}$$

where:

- S_t is the stickiness of a tentacle to a test probe in the absence of discharging cnidae, expressed in mgf;
- i_m is the intrinsic adherence of mastigophores, expressed as mgf/mastigophore;
- n_m is the number of discharged mastigophores retained on the probe;
- i_s is the intrinsic adherence of spirocysts, expressed as mgf/spirocyst;
- n_s is the number of discharged spirocysts retained on the probe.

Stickiness of tentacles (S_t). We sought to determine the contribution of tentacle surface mucus to adhesive force by measuring adhesive force in the absence of discharging cnidae. Because external calcium is required for cnida discharge in sea anemones (Blanquet, 1970; Lubbock and Amos, 1981; Thorington and Hessinger, 1992; Watson and Hessinger, 1994b), we attempted to inhibit discharge by either pre-treating anemones for 90 min in Ca-free

ASW or by following the pre-incubation in Ca-free ASW or ASW with 10% formalin for 5 min at 4°C or at room temperature before measuring adhesive force and cnida discharge. Adhesive force measurements were made using probes coated with either 30 or 75% (w/v) gelatin. After measuring adhesive force, the number of discharged mastigophores and spirocysts adhering to the test probes was determined. The data are averages of several experiments with six replicate measurements for each experiment.

Intrinsic adherence of spirocysts (i_s). To calculate the value of i_s, we measured adhesive force in the absence of contributing mastigophores using 75% gel-coated probes. Since the relatively hard surface of 75% probes makes them impenetrable to the everting tubules of most discharging mastigophores (Thorington and Hessinger, 1990), the few attached mastigophores are assumed to make no contribution to the measured adhesive force. The value of i_s was calculated from the following, modified version of Equation 1, using 75% probes in the presence of various chemosensitizers:

$$\text{Adhesive force} = S_t + (i_s)(n_s) \quad \text{(Equation 2)}$$

Experiments were performed at the concentrations of sensitizers giving maximal effects (EC_{100}) and half-maximal effects ($K_{0.5}$) on dose-responses. Sensitizers exhibiting bi- or tri-modal dose-responses (Thorington and Hessinger, 1990) were tested at each of the EC_{100} doses and the resulting values of i_s were averaged.

Intrinsic adherence of mastigophores (i_m). Values of i_m were calculated from equation 1 using the measured adhesive force, the numbers of discharged spirocysts (n_s) and mastigophores (n_m) for each tested dose of sensitizer, as well as the values of S_t and i_s, which were separately determined.

Determination of mastigophore size

Mastigophore nematocysts were discharged with 30% gel-coated probes following exposure of the Carolina strain anemones to EC_{100} doses of different sensitizers. The lengths and widths of the capsules were measured using phase-contrast optics at a final magnification of 320× (Nikon Diaphot 20 DL objective) and an ocular reticule calibrated from a stage micrometer. In addition, size comparisons of undischarged mastigophore nematocysts were made on microscope slides from squash preparations of tentacles from Miami and Carolina strains of anemones. Length and width measurements of 100 capsules were averaged for each strain.

Statistical analysis

One-way analysis of variance (ANOVA) with the Scheffe t-test was used to calculate *P*-values. A *P*-value $\leq$ 0.05 was considered to be statistically significant unless otherwise noted. Data are expressed as mean ± standard error.

Results

Tentacle stickiness (S_t)

To determine the contributions of discharging cnidae to measurements of adhesive force, it is necessary to know first the cnida-independent contributions to adhesive force, which we term tentacle stickiness (S_t). Tentacle stickiness can be measured directly from test probes in the absence of discharging cnidae. We attempted to block the discharge of cnidae and measure tentacle stickiness in several ways (Table I).

Effects of cold on cnida discharge. By chilling the ASW from room temperature to 4°C, the discharge of spirocysts onto probes coated with 30% gelatin was reduced by two thirds, the discharge of mastigophores by four fifths, and the adhesive force by about 23% (Table I). With 75% probes, chilling reduced the discharge of spirocysts onto probes by two thirds, while the number of discharged mastigophores retained on the probe was unchanged, but low, since the probes were too hard for discharging mastigophores to penetrate (Thorington and Hessinger, 1990). The adhesive force measured by 75% probes also remained unchanged, which is qualitatively consistent with our view that mastigophores, and not spirocysts, are the predominant cnida contributing to adhesive force (Thorington and Hessinger, 1990). At 4°C, 75% probes recorded significantly lower adhesive force than 30% probes despite equal numbers of discharged mastigophores and 67% more spirocysts. This suggests that the mastigophores on 30% probes contribute to adhesive force, whereas mastigophores on 75% probes do not.

Effects of low calcium on cnida discharge. Anemones, exposed for 90 min to Ca-free ASW at 4°C or exposed to 10% formalin in Ca-free ASW for 5 min at 4°C following 90 min preincubation in cold Ca-free ASW, exhibited no significant discharge of cnidae, but significant and equivalent adhesive forces (Table I). Formalin fixation does not appear to alter tentacle stickiness neither by changing the stickiness of tentacle mucus nor by altering the interaction of the tentacles with gelatin-coated test probes due to unreacted aldehyde groups, as shown by comparing the adhesive force for Ca-free ASW at 4°C with and without 10% formalin.

Effects of formalin on cnida discharge. To determine the intrinsic adherence of cnidae using Equations 1 and 2, it is first necessary to obtain a value of S_t at room temperature in ASW; the conditions used during subsequent experiments. Blocking cnida discharge with cold or with Ca-free ASW does not meet these requirements. We,

Table I

Cnida-independent contribution to adhesive force

Treatment	30% Probes			75% Probes		
	Mastig.[b]	Spiro.[c]	Ad. force[d]	Mastig.[b]	Spiro.[c]	Ad. force[d]
ASW:[a]						
22–24°C	88 ± 12	93 ± 3	56 ± 1	19 ± 3	170 ± 32	34 ± 1
4°C	19 ± 2	36 ± 6	43 ± 5	19 ± 0.2	60 ± 14	34 ± 2
Ca-free ASW:[e]						
4°C	4 ± 2	0	34.0 ± 0.7	5 ± 3	0	28.0 ± 3
4°C, formalin	1 ± 0.5	0	34.9 ± 0.5	2 ± 0.8	0	28.2 ± 7
ASW:[f]						
22–24°C, formalin	0	0.7 ± 0.1	34.6 ± 0.7	0.4 ± 0.1	0.5 ± 0.1	32.9 ± 0.3

[a] Anemones were pretreated in ASW at either room temperature (22–24°C) or 4°C for 10 min before being tested with either 30% or 75% probes (n = 20).

[b] Mean number of discharged mastigophores ± SEM.

[c] Mean number of discharged spirocysts ± SEM.

[d] Mean adhesive force, mgf ± SEM.

[e] Anemones were pretreated in Ca-free ASW for 90 min before being tested or before being exposed to 10% formalin in Ca-free ASW for 5 min at 4°C and then tested (n = 31).

[f] Anemones were pretreated in ASW for 90 min and then exposed to 10% formalin in ASW for 5 min at room temperature (22–24°C) before being tested (n = 22).

therefore, used formalin-fixation in ASW at room temperature both to preserve tentacle stickiness while also blocking cnida discharge (Table I). Thus, the measured stickiness of tentacles (S_t) to 30% probes in ASW at room temperature averages 34.6 mgf (339.1 μN), which is only 5% higher than to 75% probes (Table I).

Intrinsic adherence of spirocysts (i_s)

The contribution of discharging spirocysts to adhesive force was measured directly using 75% gelatin-coated probes. Such probes permit the adhesive tubules of discharging spirocysts to attach to the probe surface, but prevent most of the everting tubules of discharging mastigophores from penetrating (Table II; Thorington and Hessinger, 1990). Using EC_{100} doses of glycine, histamine, proline, and NANA (Thorington and Hessinger, 1990), we measured the adhesive force and counted the numbers of mastigophores and spirocysts attached to 75% probes (Table II).

The number of mastigophores retained on 75% probes averaged approximately 20% of the number retained on 30% probes under similar test conditions (Thorington and Hessinger, 1990). If we assume that the contribution to adhesive force of the relatively few, retained mastigophores on 75% probes is negligible (Table I), then the maximum contribution to adhesive force of the discharged spirocysts can be calculated from Equation 2 by subtracting the contribution of S_t (*i.e.*, 32.9 ± 0.3 mgf or 322.4 μN; Table I). Dividing the spirocyst contribution to adhesive force by the number of discharged spirocysts retained on the probe yields the intrinsic adherence of spirocysts (i_s) for each test condition (Table II). The value of i_s for spirocysts discharged from non-chemosensitized animals (*i.e.*, from Type C CSCCs) is 0.006 mgf (0.059 μN), which is lower than the average value of 0.016 mgf (0.157 μN) from chemosensitized animals (*i.e.*, from both Type B and Type C CSCCs in a ratio of about 1:1; Table II). Furthermore, the value of i_s appears to be independent of the kind of chemosensitizing agent used.

Intrinsic adherence of mastigophores (i_m)

Because we have no way of nulling the contribution of discharging spirocysts or of specifically blocking only spirocyst discharge, the contribution of discharging mastigophores cannot be directly measured as it was for spirocysts. Thus, it is necessary to calculate the contribution of discharging mastigophores using indirect approaches. This can be done by (i) relating the number of discharged mastigophores to adhesive force and graphically determining the mean intrinsic adherence from the slope of such a relationship, and by (ii) calculating i_m from equation 1 after obtaining values for all the other parameters of the equation.

Mean intrinsic adherence. The mean intrinsic adherence of discharging mastigophores can be approximated from the slope of the measured adhesive force plotted against the number of discharged mastigophores for sensitizing doses of chemosensitizers (Fig. 2). The number

of discharged mastigophores correlates directly and linearly to adhesive force. The least-squared, best-fit line for these data, when extrapolated, intercepts the abscissa at 39.9 mgf (391.0 μN). We interpret this intersection to indicate that the measured adhesive force consists of two components: one, up to about 39.9 mgf, independent of discharging mastigophores, including contributions from the stickiness of tentacles (34.6 mgf or 339.1 μN; Table I) and from discharging spirocysts; and the other dependent upon the discharging mastigophores. From the slope of this extrapolated line, we calculate that the mean intrinsic adherence of all discharging mastigophores (*i.e.*, from both Type B and Type C CSCCs) is 0.18 mgf (1.76 μN); a value more than ten times that of the average value of i_s (Table II).

Calculating i_m from Equation 1. Using the values determined for the inherent stickiness of the tentacle (S_t; Table I) and the average intrinsic adherence of the spirocysts (i_s; Table II) along with previously published values of adhesive force and of the total number of discharged spirocysts (n_s) and mastigophores (n_m) from dose-responses to various chemosensitizers (Thorington and Hessinger, 1990), it is possible to calculate the values of i_m (Eq. 1). We find that i_m varies three-fold and inversely with changes in the number of discharging mastigophores, regardless of chemosensitizer used. Overall, the values for i_m vary nonlinearly from a maximum of about 0.45 mgf (or 4.41 μN)/cnida to a minimum of about 0.15 mgf (or 1.47 μN)/cnida over the range of 50 to 250 discharged mastigophores, respectively (Fig. 3A). For each of the chemosensitizers tested, with the possible exception of proline (Fig. 3E), the shape of the i_m-discharge relationship is a declining hyperbola (Fig. 3B-D). The highest i_m values correspond to mastigophores discharged from Type C CSCCs in control anemones (Fig. 3, solid symbols), while lower i_m values correspond to mastigophores discharged from a combination of Type B and Type C CSCCs in chemosensitized anemones. The declining hyperbolic shape of the i_m-discharge relationship suggests either physical changes to the tentacle or mastigophores during discharge, which affect i_m values, or a heterogeneity in i_m values among different kinds of responding CSCCs.

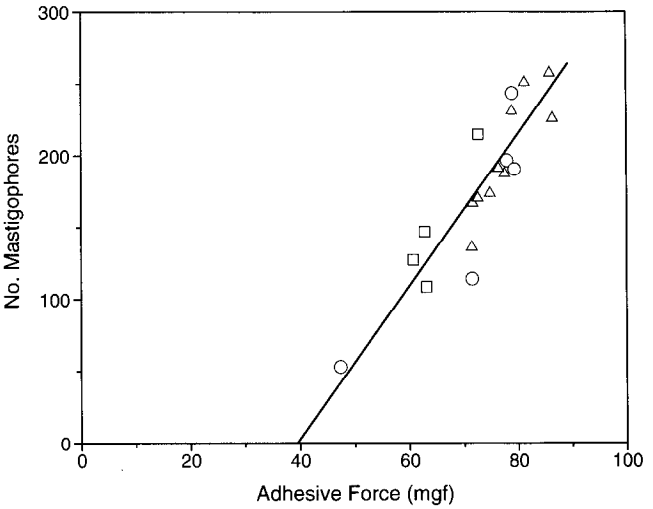

Figure 2. Number of discharged mastigophores correlated to measured adhesive force. The number of mastigophores discharged and the measured adhesive force (mgf) are indicated on the ordinate and abscissa, respectively, from anemones exposed to sensitizing doses of NANA (triangles), glycine (circles), and histamine (squares). The extrapolated least-squares fit line intercepts the abscissa. Each data point is the mean of four experiments, each comprised of six tentacle readings (R = 0.89).

Table II

Intrinsic adherence of spirocysts using 75% probes

Agonist	Mastig.[a] (n_m)	Ad. force[b] (mgf)	Spiro.[c] (n_s)	Intr. adh.[d] (i_s)
Control[e]	19 ± 3	34 ± 1	170 ± 32	0.006
Glycine (5.4×10^{-12} M)[f]	1 ± 0.4	39 ± 3	371 ± 49	0.016
Histamine[f] (10^{-12} M)	14 ± 8	32 ± 2	308 ± 13	−0.003
(9.7×10^{-10} M)	28 ± 4	37 ± 7	214 ± 69	0.019
Proline[f] (3.2×10^{-9} M)	50 ± 6	42 ± 3	343 ± 22	0.026
(1.7×10^{-7} M)	29 ± 10	39 ± 4	382 ± 35	0.016
NANA[f] (5×10^{-9} M)	19 ± 3	38 ± 3	281 ± 27	0.018
(8×10^{-8} M)	18 ± 5	39 ± 2	266 ± 56	0.023
(10^{-5} M)[g]	24 ± 2	41 ± 4	368 ± 34	0.022
			Average	0.016 ± 0.002

[a] Mean number of discharged mastigophores ± SEM (n = 5).
[b] Mean adhesive force ± SEM (n = 10).
[c] Mean number of discharged spirocysts ± SEM (n = 10).
[d] Intrinsic adherence of discharged spirocysts calculated from listed mean values of response parameters using Eq. 2 and a value of 32.9 mgf for S_t (Table I).
[e] Response parameters measured in ASW.
[f] Molarity of sensitizer producing half-maximal discharge of spirocysts, $K_{0.5}$.
[g] Molarity of sensitizer producing maximal discharge of spirocysts, EC_{100}.

Models of changes in intrinsic adherence of discharging mastigophores

To account for the declining hyperbolic shape of the i_m-discharge plot, we propose and test three models. Physical models such as our lateral pressure model and our capsule size model attempt to explain the shape of the i_m-discharge plot in terms of physical mechanisms. Models of fixed heterogeneity, such as our dilution/recruitment

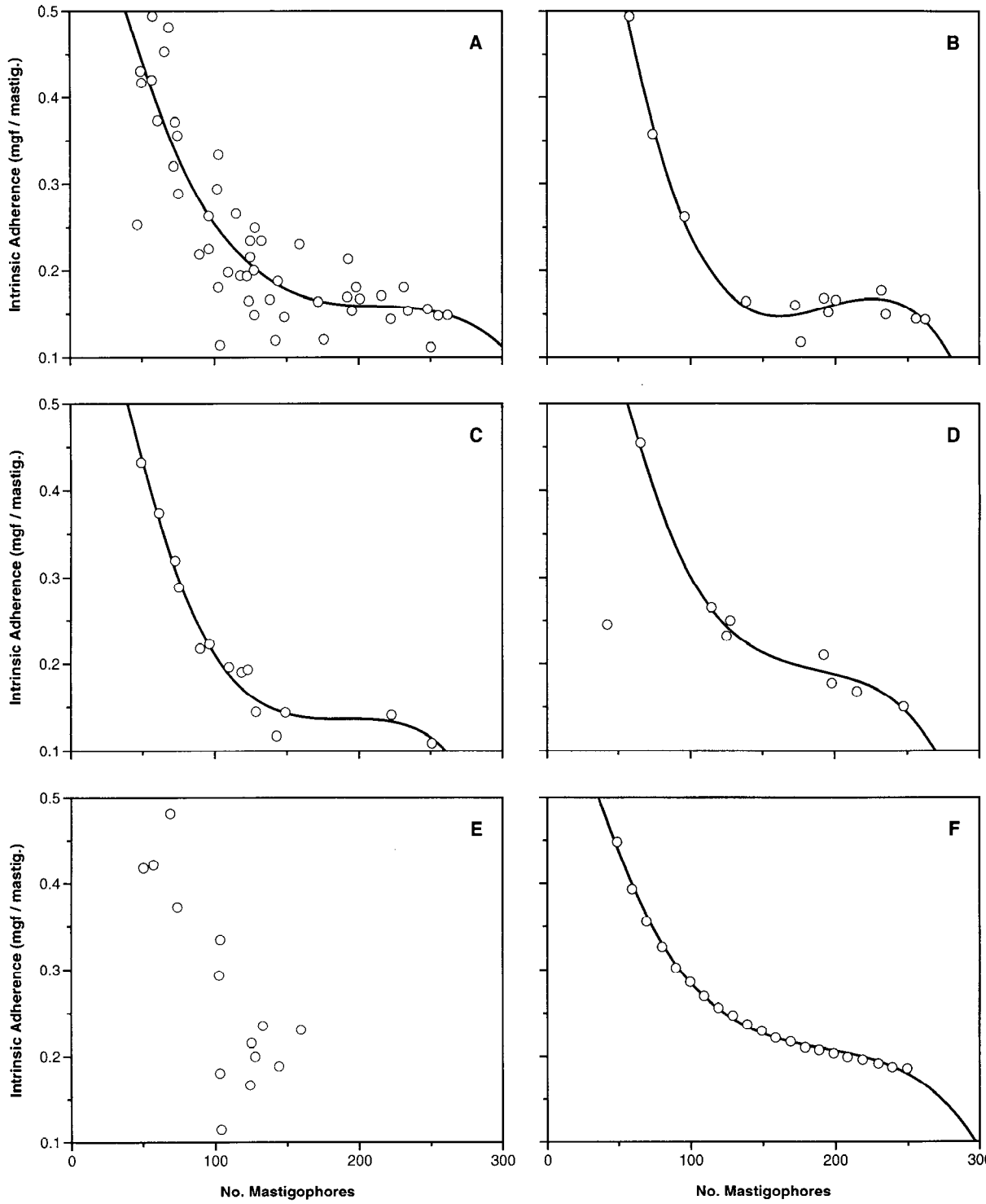

Figure 3. i_m-discharge plots of mastigophore intrinsic adherence (i_m) as a function of the number of discharged mastigophores. A. The i_m-discharge plot of i_m values calculated from Eq. 1 using data pooled from dose-responses to NANA, histamine, glycine, proline (open symbols), and ASW controls (solid symbols). Curved line is a best-fit third-degree polynomial ($R^2 = 0.71$). B. The i_m-discharge plot from dose-responses to NANA of adhesive force and numbers of discharged mastigophores and spirocysts ($R^2 = 0.99$). C. The i_m-discharge plot from dose-responses to histamine of adhesive force and numbers of discharged mastigophores

model, attempt to explain the i_m-discharge plot in terms of different mastigophore-bearing CSCCs characterized by distinct and constant i_m values.

Lateral pressure model. For our lateral pressure model, we propose that the intrinsic adherence of discharging cnidae arises in significant part from lateral pressure and 9,,,R,I0xcompressive forces generated within the ectoderm by the turgid state of adjacent undischarged cnidae. A decrease in that pressure, due to the contraction of cnidae at discharge, could cause a discharge-dependent decrease in measured i_m values in proportion to the number of discharging cnidae, such as observed in Figure 3. Indeed, we observe a 29% decrease ($P < 0.0001$) in the mean width of mastigophores after discharge; much greater than the decrease in capsule lengths (2.6%; Table III). Such changes reflect a 50% decrease in calculated volume (Thomason, 1988) during discharge of mastigophores from the tentacles of Carolina strain anemones.

The lateral pressure model is difficult to test experimentally, but, as a physical model for changes in i_m, it should apply invariantly. Validation or invalidation of this model depends upon the consistency or inconsistency of the inverse correlation of changing i_m values to the numbers of discharging mastigophores. We note several such deviations from the inverse correlation between i_m and discharge. While the i_m-discharge plots of NANA- and histamine-sensitized anemones generally show consistency of correlation (Fig. 3B, C), the plot of glycine-sensitized discharge shows one or two exceptions (Fig. 3D), and the plot of proline-sensitized discharge shows several exceptions (Fig. 3E). We conclude that while mastigophore capsules contract significantly during discharge, such changes do not cause, but may contribute to, the measured decreases in i_m values with increasing numbers of discharging mastigophores.

Capsule size model. Cnida capsules from anthozoans are prolate ellipsoidal in shape. In our capsule size model, we state that large diameter mastigophores require more force to dislodge them from the tentacle than smaller diameter mastigophores. It then follows that differences in the cross-sectional diameter of mastigophores discharged from Type B and Type C CSCCs will account for the observed differences of i_m. Thus, the capsule size model predicts that high-i_m mastigophores discharged from Type C CSCCs under control conditions will have wider capsules than mastigophores discharged from chemosensitized Type B CSCCs.

Table III

Dimensions of discharged and undischarged mastigophore capsules

Agonist	Length (μm)	Width (μm)
A. Discharged, Carolina Strain[a]		
Control	31.6 ± 0.2	5.1 ± 0.1
Glycine (5.4×10^{-12} M)[b]	30.5 ± 0.3	5.0 ± 0.1
Histamine[b]		
(9.7×10^{-10} M)	30.8 ± 0.3	5.1 ± 0.1
(10^{-7} M)	30.2 ± 0.2	5.0 ± 0.1
Avg.	30.5 ± 0.2	5.1 ± 0.1
N-acetylneuraminic Acid[b]		
(5×10^{-9} M)	32.6 ± 0.3	5.2 ± 0.1
(8×10^{-8} M)	32.8 ± 0.5	5.0 ± 0.1
(10^{-5} M)[c]	33.2 ± 0.2	5.3 ± 0.1
Avg.	32.8 ± 0.2	5.2 ± 0.1
B. Undischarged[d]		
Carolina Strain	32.4 ± 0.5	7.2 ± 0.3
Miami Strain	31.7 ± 0.5	6.0 ± 0.3

[a] Measurements represent the mean of 100 mastigophore capsules selected at random after being discharged onto 30% gelatin-coated probes by Carolina strain anemones in response to combined chemical and mechanical stimulation.

[b] Molarity of sensitizer producing half-maximal discharge of spirocysts, $K_{0.5}$.

[c] Molarity of sensitizer producing maximal discharge of both spirocysts and mastigophores, EC_{100}.

[d] Measurements represent the mean of 102 mastigophore capsules selected haphazardly from tentacle squash preparations.

We measured the dimensions of mastigophore capsules discharged onto test probes from control anemones (*i.e.*, discharged from Type C CSCCs) and from anemones chemosensitized at $K_{0.5}$ and EC_{100} levels of various sensitizers (*i.e.*, discharged from both Type B and Type C CSCCs in ratios ranging from 2:1 to 5:1, respectively). We discern no significant differences between the mean widths of discharged mastigophores from control and chemosensitized anemones (Table III). We conclude that the measured three-fold difference in i_m values cannot be accounted on the basis of differences in the sizes of discharged mastigophore capsules. We also conclude that mastigophores discharged from Type B CSCCs and from Type C CSCCs do not differ in terms of capsule size.

In addition, undischarged mastigophores from tentacle squash preparations of Carolina strain anemones used in the present study are significantly wider ($P < 0.001$) and slightly longer ($P < 0.05$; Table III) than the undischarged capsules from monoclonal Miami strain anemones used

and spirocysts ($R^2 = 0.98$). D. The i_m-discharge plot from dose-responses to glycine of adhesive force and numbers of discharged mastigophores and spirocysts ($R^2 = 0.99$). E. The i_m-discharge plot from dose-responses to proline of adhesive force and numbers of discharged mastigophores and spirocysts. F. Simulation of dilution/recruitment model in which are plotted the weighted average i_m values calculated from Eq. 3 in which the contributions of mastigophores discharged from Type C CSCCs are assumed to be constant.

in a previous study (Geibel *et al.,* 1988). While undischarged nematocysts from the monoclonal Carolina strain measure larger than those from the Miami strain, the mean i_m value calculated for Carolina strain nematocysts of 0.18 mgf (1.76 μN; Fig. 2) is the same as that calculated for the narrower Miami strain nematocysts of 0.17 mgf (1.67 μN; Geibel *et al.,* 1988). Because differences in capsular widths between strains do not seem to give rise to significant differences in mean i_m values and because the measured differences in i_m values of nematocysts discharged from control and chemosensitized anemones cannot be accounted for on the basis of differences in capsular widths, we conclude that differences in nematocyst diameter, of the range encountered in the present study, do not significantly affect the intrinsic adherence of mastigophores.

Dilution/recruitment model. We can reproduce the general shape of the i_m-discharge plot using a model in which a constant number of high-i_m mastigophores discharged from Type C CSCCs are numerically diluted by increasing numbers of lower-i_m mastigophores discharged from Type B CSCCs. We call our model the dilution/recruitment model. Our model can be expressed in the following equation for the weighted average i_m value:

$$i_m = [(n_{mC})(i_{mC}) + (n_{mB})(i_{mB})]/n_m \quad \text{(Equation 3)}$$

in which,

- i_m is the weighted average intrinsic adherence of all discharging mastigophores;
- n_{mC} is the number of mastigophores discharged from Type C CSCCs under control conditions and assumed to be constant (50 mastigophores; see Fig. 3);
- i_{mC} is the intrinsic adherence of mastigophores discharged from Type C CSCCs under control conditions and assumed to be fixed at 0.45 mgf (Fig. 3);
- n_{mB} is the number of mastigophores discharged from Type B CSCCs and varies (*e.g.,* from 0 to 250 mastigophores);
- i_{mB} is the intrinsic adherence of mastigophores discharged from Type B CSCCs and is assumed to be fixed (*e.g.,* 0.12 mgf; Fig. 3);
- n_m is the total number of mastigophores discharged (*i.e.,* $n_m = n_{mB} + n_{mC}$).

A simulation of our dilution/recruitment model (Fig. 3F), in general, shows similarities in shape to the i_m-discharge plots sensitized by NANA (Fig. 3B), by histamine (Fig. 3C), by glycine (Fig. 3D), and by proline (Fig. 3E). The dilution/recruitment model simulates the i_m-discharge plot for glycine (Fig. 3D), except for one data point corresponding to the highest tested concentration of glycine. Our model also seems to reproduce the general shape of the i_m-discharge plots for NANA (Fig. 3B) and histamine (Fig. 3C), except that figures 3B and 3C decline more steeply than the simulated plot of the dilution model and level off at lower values. Our model simulates some of the changes in the value of i_m for proline-sensitized discharge (Fig. 3E), except at high, desensitizing levels of proline where the value of i_m declines abruptly. These i_m-discharge plots suggest that the dilution/recruitment model generally, but not fully, describes the changes in i_m caused by sensitizing with NANA, histamine, and glycine.

Discussion

The properties of cnidae

Spirocysts are "adhesive organelles" (Mariscal *et al.,* 1977). By inference, spirocysts have been reputed to be the primary contributors of cnida-mediated attachment between the tentacle and inanimate substrates: ". . . spirocysts are responsible for the adhesion of tentacles to shells" (McFarlane and Shelton, 1975); and ". . . spirocysts are the primary adhesive organelle in such situations" (*i.e.,* substrate attachment) ". . . at least in *Anemonia*" (Conklin and Mariscal, 1976). Spirocysts have also been reputed to be contributors of cnida-mediated attachment to prey: (i) ". . . spirocysts . . . discharge to hold the prey" (Williams, 1968); (ii) "It is possible that the commonly observed 'stickiness' of sea anemone tentacles when lightly contacted is largely due to the discharge of spirocysts. . . . Similarly, the initial adhesion of the tentacles of a sea anemone to an unacclimated anemone fish could also be due in large part to the discharge of spirocysts (*e.g.,* Mariscal, 1970)" (Mariscal *et al.,* 1977); and (iii) "The primary function of spirocysts is in adhesion, both to substrate materials and to food objects" (Mariscal, 1984). In contrast, the everting tubule of nematocysts penetrate and envenomate prey, but also entangle (Ewer, 1947). By inference, nematocysts seem to have been relegated to playing a minor role in mediating attachment between tentacle and target (Williams, 1968; Shelton, 1980).

Several authors have attempted to determine the roles of different kinds of nematocysts in hydrozoans by observing the cnidae deployed during the capture of prey (Ewer, 1947; Purcell, 1984; Purcell and Mills, 1988). However, few functional comparisons between discharging spirocysts and nematocysts in anthozoans have been performed (Williams, 1968; McFarlane and Shelton, 1975). Attempts to ascertain cnida function based on numbers of discharged spirocysts and nematocysts retained on targets (*e.g.,* McFarlane and Shelton, 1975) tacitly, but incorrectly, assume that the tentacle adherence for each kind of cnida are equal. We recently noted that measurements of adhesive force correlate closely to the

number of discharged mastigophores (Giebel *et al.*, 1988) and that the dose-responses of chemosensitized nematocyst discharge from Type B CSCCs correlate much more closely to the adhesive force dose-responses than to the dose-responses of chemosensitized spirocyst discharge (Thorington and Hessinger, 1990).

In acontiate sea anemones, cnida-mediated prey capture is regulated by both afferent and efferent mechanisms. Afferent mechanisms of cnidae influence detection and selection of prey and regulate the selective discharge of cnidae under a hierarchy of interactions: (i) between prey and predator (Thorington and Hessinger, 1988b); (ii) between CSCCs of the same and different tentacles (Hessinger *et al.*, 1992; Watson and Hessinger, 1994a); (iii) between remote sensory cells and the CSCCs (Mire-Thibodeaux and Watson, 1993); (iv) between the sensory supporting cells and the effector cnidocyte within the same CSCC (Watson and Hessinger, 1989); and (v) between chemo- and mechanoreceptor systems located on the same supporting cells (Watson and Hessinger, 1991). Efferent mechanisms of cnidae, on the other hand, determine the manner in which discharged cnidae are used to apprehend and retain captive prey. We distinguish at least three types of interaction between targets and cnida based on efferent mechanisms: (i) cnida penetration; (ii) cnida adhesion; and (iii) tentacle adherence. Until now, efferent mechanisms of discharging cnidae have not been directly studied nor measured.

Contributions to adhesive force

The measured adhesive force represents the force required to separate the test probe from the tentacle expressed in hybrid units of milligrams-force (mgf) or newtons (μN) and is an aggregate measure of both cnida-independent and cnida-mediated contributions (equation 1). In this report we have systematically measured and analyzed these contributions.

Cnida-independent contributions to adhesive force. The cnida-independent contribution to adhesive force was measured as the stickiness of the tentacle (S_t) to test probes in the absence of discharging cnidae (Table I). We inhibited the discharge of cnidae by using either cold (4°C) Ca-free ASW on living anemones or 10% formalin in ASW at room temperature. The two methods gave essentially equal measures of tentacle stickiness.

Tentacle stickiness appears to be a major contribution to the adhesive force measured with gelatin-coated targets. We infer that tentacle stickiness contributes to the retention of captured prey to the extent that the prey surface is smooth and adhesively interactive with the tentacle mucus.

Cnida-mediated contributions to adhesive force. Cnida-mediated contributions to adhesive force reflect both the number and the kind of discharging cnidae attaching to the target. The contribution of each kind of cnida to adhesive force is determined by opposing forces operating on the discharged cnida; one from the tentacle and another from the target. The force operating from the tentacle determines the extent to which the cnida capsule is attached to the tentacle and is referred to as tentacle adherence (Cormier and Hessinger, 1980). Likewise, the force operating from the target determines the extent to which the everted cnida tubule is attached to the target and is referred to as cnida adhesion. Under conditions in which cnida adhesion exceeds tentacle adherence, all discharged cnidae are retained on the target and the adhesive force measures the adherence of the cnidae to the tentacle.

Cnida adhesion. Factors affecting cnida adhesion are related to the nature of the everted tubule and to the physical and chemical characteristics of the target. As the hardness of the target is increased, progressively more mastigophores are retained until a point is reached beyond which progressively fewer discharging mastigophores attach to the target, due to the inability to penetrate (Thorington and Hessinger, 1990). For maximum adhesion of penetrant mastigophores, the surface of the target must be sufficiently soft to permit the everting tubule to penetrate the target, yet sufficiently hard to retain them when the target is withdrawn from the tentacle. Maximum retention of discharged mastigophores occurs with probes coated with 30–40% gelatin. Under such conditions mastigophore adhesion exceeds its adherence to the tentacle, and the contribution of mastigophores to adhesive force is a measure of tentacle adherence.

For maximum adhesion of discharged spirocysts, the target surface must be sufficiently hard that the sticky, everted tubules are not easily detached when the target is withdrawn from the tentacle. Coating test probes with 30% and higher concentrations of gelatin causes maximal retention of discharged spirocysts (Thorington and Hessinger, 1990). In addition, the surface of the target must be sufficiently reactive with the bioadhesive material of the everted spirocyst tubule for the everted tubules to attach firmly. Little is currently known about the chemistry of the spirocyst adhesive except that it binds N-acetylated sugars with high affinity (Thorington and Hessinger, 1990). In the present experiments, test probes have been constructed either to maximize or to minimize cnida adhesion. Specifically, 75% gelatin-coated probes maximize retention of spirocysts and minimize adhesion of the mastigophores, while 30% probes maximize the cnida adhesion of both kinds (Thorington and Hessinger, 1990).

Tentacle adherence. Several factors potentially influence tentacle adherence; some passive and some active. Passive factors potentially include considerations of the size and shape of the cnida capsules, of the lateral pressure exerted

by the adjacent epithelium, as well as any possible cytoskeletal elements, such as the participation of anchoring fibrillar baskets and hemidesmosomal junctions, as occur in hydrozoan nematocytes (Cormier and Hessinger, 1980; Wood and Novak, 1982). In this paper, we show no evidence indicating that such passive factors as capsule size or lateral pressure directly influence tentacle adherence in *A. pallida* (Table III). Active factors potentially involve the contraction of intracellular cytoskeletal elements, such as has been proposed for cnidocytes of *Physalia* (Hessinger and Ford, 1988), and the constriction of adjacent accessory cells, such as the supporting cells that surround cnidocytes in anemone feeding tentacles. Our model indicates that in anemone feeding tentacles, cnidocytes function as passive effectors, triggered to discharge by contracting supporting cells. According to this model, tentacle adherence is produced by a sustained contraction of the supporting cells within the CSCCs.

In our measures of tentacle adherence, it is necessary that all of the discharged cnidae be retained on the test probe following the separation of the target from the tentacle (*i.e.,* cnida adhesion exceeds tentacle adherence). When these conditions are met, as they are when using 30–40% gelatin-coated probes (Thorington and Hessinger, 1990), and when the tentacle stickiness is known (Table I), then the measurement of adhesive force can be used to calculate tentacle adherence. In addition, when the numbers of different discharged cnidae are also known, then it is possible to calculate the contribution of individual cnidae to tentacle adherence—a unitary parameter termed the intrinsic adherence.

Intrinsic adherence. The intrinsic adherence is a limit or unitary parameter of discharged cnidae that represents the adhesive force contributed by a single discharged cnida against a target probe. The intrinsic adherence of each kind of cnida is a biologically relevant parameter that bears on its role in holding struggling prey onto the tentacle.

Contributions of spirocysts to adhesive force. Ours is the first measurement of the intrinsic adherence of either spirocysts or nematocysts. If we assume that the nematocysts retained on probes coated with 75% gelatin do not contribute to adhesive force, then the intrinsic adherence of discharged spirocysts (i_s) in *A. pallida* tentacles measures about 0.016 mgf (0.157 μN) for spirocysts from Type B CSCCs, and less than half that value for spirocysts from Type C CSCCs. However, our measures of i_s are overestimates due to our simplifying assumption that the mastigophores attached to 75% probes do not contribute to the measured adhesive force. Our assumption is not likely correct, because the attached mastigophores would not be retained if they were loosely attached. In other words, if mastigophores are retained on 75% probes, then their cnida adhesion exceeds their tentacle adherence and such mastigophores contribute to the measured adhesive force in proportion to the product of their numbers and intrinsic adherence. If we assume that the intrinsic adherence of spirocysts is insignificant and then calculate the value of i_m on 75% probes from equation 1 using the data on Table II (excluding glycine), the resulting average value of i_m is 0.19 mgf (1.86 μN). This value is consistent with the mean value of i_m in Figures 2 and 3. Thus, in our judgment, the value of i_s is negligible and spirocysts do not directly contribute to adhesive force.

Contributions of mastigophores to adhesive force. In the present study, using the Carolina strain of *A. pallida,* we verify our earlier report with the Miami strain of the same species (Geibel *et al.,* 1988) that the adhesive force measurements correlate positively with the number of discharged mastigophores (Fig. 2). From the slope of the linear correlation between the number of discharged mastigophores and the measured adhesive force, the mean i_m value is 0.18 mgf (1.76 μN) for the Carolina strain, and for the Miami strain it is 0.17 mgf (1.67 μN; Geibel *et al.,* 1988). The i_m-discharge curves (*i.e.,* i_m plotted against number of discharging nematocysts; Fig. 3) indicate that the values of i_m vary inversely and hyperbolically with the number of discharging nematocysts, ranging from a maximum value of about 0.45 mgf (or 4.41 μN)/mastigophore at low numbers of discharged mastigophores from Type C CSCCs in control anemones to 0.15 mgf (or 1.47 μN)/mastigophore at relatively high numbers of mastigophores from Type B plus Type C CSCCs in chemosensitized anemones. These values of i_m are very high in relation to the minimal or negligible values estimated for i_s. It seems reasonable that the intrinsic adherence of mastigophores should be high in relation to the values for spirocysts, given that the capsules of mastigophores are thick-walled and multi-layered (Blake *et al.,* 1988), whereas the capsules of spirocysts are thin-walled and single-layered (Mariscal *et al.,* 1976).

The declining i_m-discharge plot. We find that the observed three-fold change in i_m does not correlate with variations in capsule diameter nor is it caused by decreases in lateral pressure from the discharge of adjacent cnidae (Table III). In general, the decline in i_m with increasing numbers of discharging mastigophores is simulated by the recruitment/dilution model in which there are two populations of discharging mastigophores characterized by fixed high and low i_m values originating from Type C and Type B CSCCs, respectively. Under control (*i.e.,* non-chemosensitized) conditions and under desensitizing concentrations of chemosensitizer, a constant number of mastigophores discharged from Type Cs predominate, causing the measured i_m to be high (*i.e.,* about 0.45 mgf or 4.41 μN). With sensitizing concentrations of chemo-

sensitizer, the discharge of progressively greater numbers of mastigophores from Type B CSCCs occurs, causing the value of i_m to decrease asymptotically to about 0.15 mgf (1.47 μN). The general fit of the dilution/recruitment model with our data also supports our designation of Type B and Type C CSCCs. The recruitment/dilution model, however, fails to account for the steepness of the decline of the i_m-discharge plot for NANA and histamine (Fig. 3B, C) and does not account for some of the i_m values plotted for proline (Fig. 3E). These deviations from the recruitment/dilution model suggest that the contribution to adhesive force of mastigophores discharging from Type C CSCCs may not be constant as we have assumed.

Roles of mastigophores and spirocysts in the capture of prey

Our findings indicate that discharging mastigophores, but not spirocysts, secure gelatin-coated targets to the feeding tentacles of *A. pallida.* From these findings, we infer that penetrable prey are secured by similar means. Our findings also indicate that the stickiness of the tentacle surface contributes significantly to the measured adhesive force. Thus, tentacle mucus likely contributes to the retention of small prey to the tentacles.

While undischarged spirocysts are about three times more numerous than mastigophores on the tentacles of *A. pallida,* the value of i_m for Type C CSCCs is at least 30 times higher than the maximum estimate of i_s. Because the value of i_s is low (Table II) to negligible in relation to the value of i_m, and because the dose-responses of discharging spirocysts do not coincide with the dose-responses of adhesive force (Thorington and Hessinger, 1990), we conclude that discharging spirocysts do not directly secure struggling prey to the tentacle. If spirocysts participate in securing prey, their action is likely indirect or restricted to very small prey. The data on Table II for 75% probes may be interpreted to suggest that spirocysts indirectly contribute to the retention of prey by assisting the attachment of everting mastigophore tubules to impenetrable surfaces, as suggested by McFarlane and Shelton (1975). We speculate that discharging spirocysts may also assist in prey capture by interfering with and fouling the swimming appendages of prey.

It is possible to estimate the minimum number of mastigophores and spirocysts needed to retain struggling prey to the tentacle, if the dynamics of swimming prey are known. The principal prey of small acontiate anemones, such as *A. pallida,* are small swimming crustaceans. The sole prey of laboratory-reared *A. pallida* used in the present study are freshly hatched *Artemia salina* nauplii (mass of approximately 0.01 mg). Based upon high-speed motion analysis of swimming *Artemia* nauplii, we calculate that they exert a maximum combined force (viscous drag plus inertial force) of approximately 0.2 μN per power stroke while swimming at an average velocity of 1.5 mm/s and a stroke frequency of 7.5 Hz (unpub.). We conclude that a single discharged mastigophore (i_m values of 4.41 to 1.47 μN) is more than sufficient to secure an individual, struggling *Artemia* nauplius to the tentacle of *A. pallida.* On the other hand, it is not conceivable that a single discharged spirocyst could tether a struggling nauplius unless it also fouls a swimming appendage. In our judgment, more than one spirocyst would be needed. For larger prey, such as *Cyclops,* with a mass of 1.025 mg, which exerts 6.6 μN of force per power stroke (Alcaraz and Strickler, 1988), at least two mastigophores from Type C CSCCs or five from Type B CSCCs, or some combination thereof, would be needed. It seems unlikely, however, that spirocysts alone would be capable of capturing and retaining such a massive prey without the involvement of mastigophores.

Conclusions

1. The ability of discharging cnidae to secure captured prey on tentacles relies upon two properties common to all cnidae: the adhesion of the cnida tubule to the prey and the adherence of the cnida capsule by the tentacle. This report provides the first measure of tentacle adherence and of the contributions of specific types of cnidae to adhesive force.

2. The upper limit of the calculated intrinsic adherence of spirocysts (i_s) is between 0.006 to 0.016 mgf (0.059 to 0.157 μN). The true value of i_s is likely much smaller to insignificant.

3. The value of i_m for a mastigophore discharged from Type C CSCCs under non-chemosensitized conditions is approximately 0.45 mgf (4.41 μN). We are not yet able to obtain accurate values of i_m for mastigophores discharged from Type B CSCCs, but a lower limit is in the range of 0.15 mgf (1.47 μN).

4. The mean value of i_m does not vary with different kinds of chemosensitizers, but varies inversely with the total number of discharging mastigophores. The dilution/recruitment model based upon the discharge of a variable number of mastigophores from low-i_m Type B CSCCs and a fixed number of mastigophores from high-i_m Type C CSCCs, in general, simulates the inverse correlation between i_m and number of discharging nematocysts.

5. The contribution of tentacle stickiness (S_t) to adhesive force is significant, suggesting that tentacle stickiness contributes, although non-selectively, to the retention of captured prey.

6. Mastigophores discharged from Type C CSCCs under control conditions are the same size as mastigophores

discharged from Type B CSCCs during chemosensitization, and differences in mean i_m values do not appear to be due to differences in the mean diameters of capsules nor to decreases in capsule volume during discharge.

7. The number of discharging mastigophores and spirocysts decreases by four-fifths and two thirds, respectively, at 4°C, and is completely blocked in Ca-free ASW.

Acknowledgments

We thank Drs. Daphne Fautin and Richard Mariscal for helpful comments about the manuscript. Supported in part by NSF grant MCB-8919269.

Literature Cited

Alcaraz, M., and J. R. Strickler. 1988. Locomotion in copepods: patterns of movements and energetics of *Cyclops. Hydrobiologia* **167/168:** 409–414.

Blake, A. S., R. S. Blanquet, and G. B. Chapman. 1988. Fibrillar ultrastructure of the capsular wall and intracapsular space in developing nematocysts of *Aiptasia pallida* (Cnidaria: Anthozoa). *Trans. Am. Microsc. Soc.*, **107:** 217–231.

Blanquet, R. 1970. Ionic effects on discharge of the isolated and *in situ* nematocysts of the sea anemone *Aiptasia pallida:* a possible role of calcium. *Comp. Biochem. Physiol.* **35:** 451–461.

Conklin, E. J., and R. N. Mariscal. 1976. Increase in nematocyst and spirocyst discharge in a sea anemone in response to mechanical stimulation. Pp. 549–558 in *Coelenterate Ecology and Behavior,* G. O. Mackie, ed. Plenum Press, New York.

Cormier, S. M., and D. A. Hessinger. 1980. Cellular basis for tentacle adherence in the Portuguese Man-of-War (*Physalia physalia*). *Tissue Cell* **12:** 713–721.

Dorgan, L., and D. A. Hessinger. 1984. GRAFPAC, a Graphics and Format Package for the Apple II+ (IIe) Computer. Copyright 1984.

Ewer, R. F. 1947. On the functions and mode of action of the nematocysts of hydra. *Proc. Zool. Soc. London* **117:** 365–376.

Geibel, G., G. Thorington, and D. A. Hessinger. 1988. Control of cnida discharge: II. Microbasic p-mastigophore nematocysts are regulated by two classes of chemoreceptors. *Biol. Bull.* **175:** 132–136.

Hessinger, D. A., and M. T. Ford. 1988. Ultrastructure of the small cnidocyte of the Portuguese Man-of-War (*Physalia physalis*) tentacle. Pp. 75–94 in *The Biology of Nematocysts.* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Hessinger, D. A., C. Fulenwider, M. Shah, and W. H. Fletcher. 1992. Possible cnidarian homolog of connexin 43 (Cx43) gap junctions in sea anemones. ASCB abstract H60, Denver, CO.

Lenhoff, H. M., and W. Haegy. 1977. Aquatic invertebrate model systems for study of receptor activation and evolution of receptor proteins. *Ann. Rev. Pharmacol. Toxicol.* **17:** 243–258.

Lubbock, R., and W. B. Amos. 1981. Removal of bound calcium from nematocyst contents causes discharge. *Nature* **290:** 500–501.

Mariscal, R. N. 1970. An experimental analysis of the protection of *Amphiprion xanthurus* Cuvier & Valenciennes and some other anemone fishes from sea anemones. *J. Exp. Mar. Biol. Ecol.* **4:** 134–149.

Mariscal, R. N. 1974. Nematocysts. Pp. 129–178 in *Coelenterate Biology: Reviews and New Perspectives,* L. Muscatine and H. M. Lenhoff, eds. Academic Press, New York.

Mariscal, R. N. 1984. Cnidaria: Cnidae. Pp. 57–68 in *Biology of the Integument. Vol. I. Invertebrates.* J. Bereiter-Hahn, A. G. Maltolsy, and K. S. Richards eds. Springer-Verlag, Berlin.

Mariscal, R. N., C. H. Bigger, and R. B. McLean. 1976. The form and function of cnidarian spirocysts. I. Ultrastructure of the capsule exterior and relationship to the tentacle sensory surface. *Cell Tissue Res.* **168:** 465–474.

Mariscal, R. N., R. B. McLean, and C. Hand. 1977. The form and function of cnidarian spirocysts. III. Ultrastructure of the thread and function of spirocysts. *Cell Tissue Res.* **178:** 427–433.

McFarlane, I. D., and G. A. B. Shelton. 1975. The nature of the adhesion of tentacles to shells during shell-climbing in the sea anemone *Calliactis parasiticia* (Couch). *J. Exp. Mar. Biol. Ecol.* **17:** 177–186.

Miller, F., Jr., 1959. P. 58 in *College Physics,* Harcourt, Brace and World, New York.

Mire-Thibodeaux, P. M., and G. M. Watson, 1993. Direct monitoring of intracellular calcium ions in sea anemone tentacles suggests regulation of nematocyst discharge by remote, rare epidermal cells. *Biol. Bull.* **185:** 335–345.

Purcell, J. E. 1984. The functions of nematocysts in prey capture by epipelagic siphonophores (Coelenterata, Hydrozoa). *Biol. Bull.* **166:** 310–327.

Purcell, J. E., and C. E. Mills. 1988. The correlation between nematocyst types and diets in pelagic hydrozoa. Pp. 463–485 in *The Biology of Nematocysts,* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Shelton, G. A. B. 1980. Anthozoa. Pp. 203–242 in *Electrical Conduction and Behavior in Simple Invertebrates,* G. A. B. Shelton, ed. Oxford University Press, Oxford, UK.

Thomason, J. C. 1988. The allometry of nematocysts. Pp. 575–588 in *The Biology of Nematocysts,* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Thorington, G. U., and D. A. Hessinger. 1988a. Control of cnida discharge: I. Evidence for two classes of chemoreceptors. *Biol. Bull.* **174:** 163–171.

Thorington, G. U., and D. A. Hessinger. 1988b. Control of cnida discharge: factors affecting discharge of cnidae. Pp. 233–253 in *The Biology of Nematocysts,* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Thorington, G. U., and D. A. Hessinger. 1990. Control of cnida discharge: III. Spirocysts are regulated by three classes of chemoreceptors. *Biol. Bull.* **178:** 74–83.

Thorington, G. U., and D. A. Hessinger. 1992. Nifedipine blocks desensitization of chemoreceptors regulating nematocyst discharge in sea anemones (*Aiptasia pallida*). *Molec. Biol. Cell* **3:** 250a.

Watson, G. M., and D. A. Hessinger. 1988. Localization of a purported chemoreceptor involved in triggering cnida discharge in sea anemones. Pp. 255–272 in *The Biology of Nematocysts,* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Watson, G. M., and D. A. Hessinger. 1989. Cnidocyte mechanoreceptors are tuned to the movements of swimming prey by chemoreceptors. *Science* **243:** 1589–1591.

Watson, G. M., and D. A. Hessinger. 1991. Chemoreceptor-mediated elongation of stereocilium bundles tunes vibration-sensitive mechanoreceptors on cnidocyte-supporting cell complexes to lower frequencies. *J. Cell. Sci.* **99:** 307–316.

Watson, G. M., and D. A. Hessinger. 1994a. Antagonistic frequency tuning of hair bundles by different chemoreceptors regulates nematocyst discharge. *J. Exp. Biol.* **187:** 57–73.

Watson, G. M., and D. A. Hessinger. 1994b. Evidence for calcium channels involved in regulating nematocyst discharge. *Comp. Physiol. Bioch.* **107A:** 473–481.

Williams, R. B. 1968. Control of the discharge of cnidae in *Diadumene luciae* (Verill). *Nature* **219:** 959–960.

Wood, R. L., and P. L. Novak. 1982. The anchoring of nematocysts in the tentacles of *Hydra. J. Ultrastruct. Res.* **81:** 104–116.